2-Styrylchromones inhibit IL-1β-induced inflammatory mediators' production in human fibroblast-like synoviocytes

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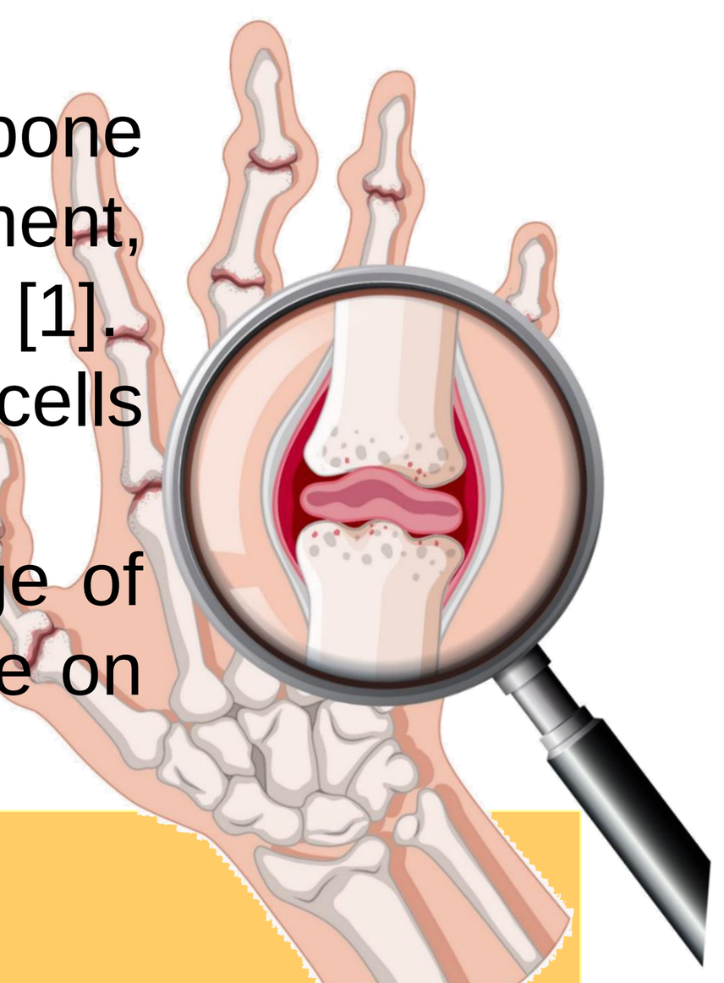
Background



Rheumatoid arthritis (RA) is a progressive, chronic, autoimmune disease, characterized by persistent synovial inflammation and irreversible cartilage and bone damage that primarily affects the synovial joints and adjacent tissues. Despite the numerous novel and differentiated molecules rising for RA treatment, presently, effective drugs that can control synovitis and catabolism in the RA process, without significant side effects and real efficacy remain an unmet need [1]. The role of fibroblast-like synoviocytes (FLS), specialized cells located in the synovium, on synovial inflammation initiation and progression, make these cells natural targets for the search of new effective molecules to stop or halt the disease progression [2].

2-Styrylchromones (2-SC) are chromone derivatives characterized by the existence of a styryl group in the chromone structure that feature a wide range of biological properties, including antioxidant and anti-inflammatory activities [3]. As far as we know 2-SC have never been investigated for their potential role on RA treatment.

Aims

The present study investigated the effect of a set of six 2-SC (figure 1) with hydroxy and methoxy substituents on the IL1β - induced increase of •NO release and iNOS expression levels in human fibroblast-like synoviocytes (HFLS), pointing the role of NF-κB activation in the process.

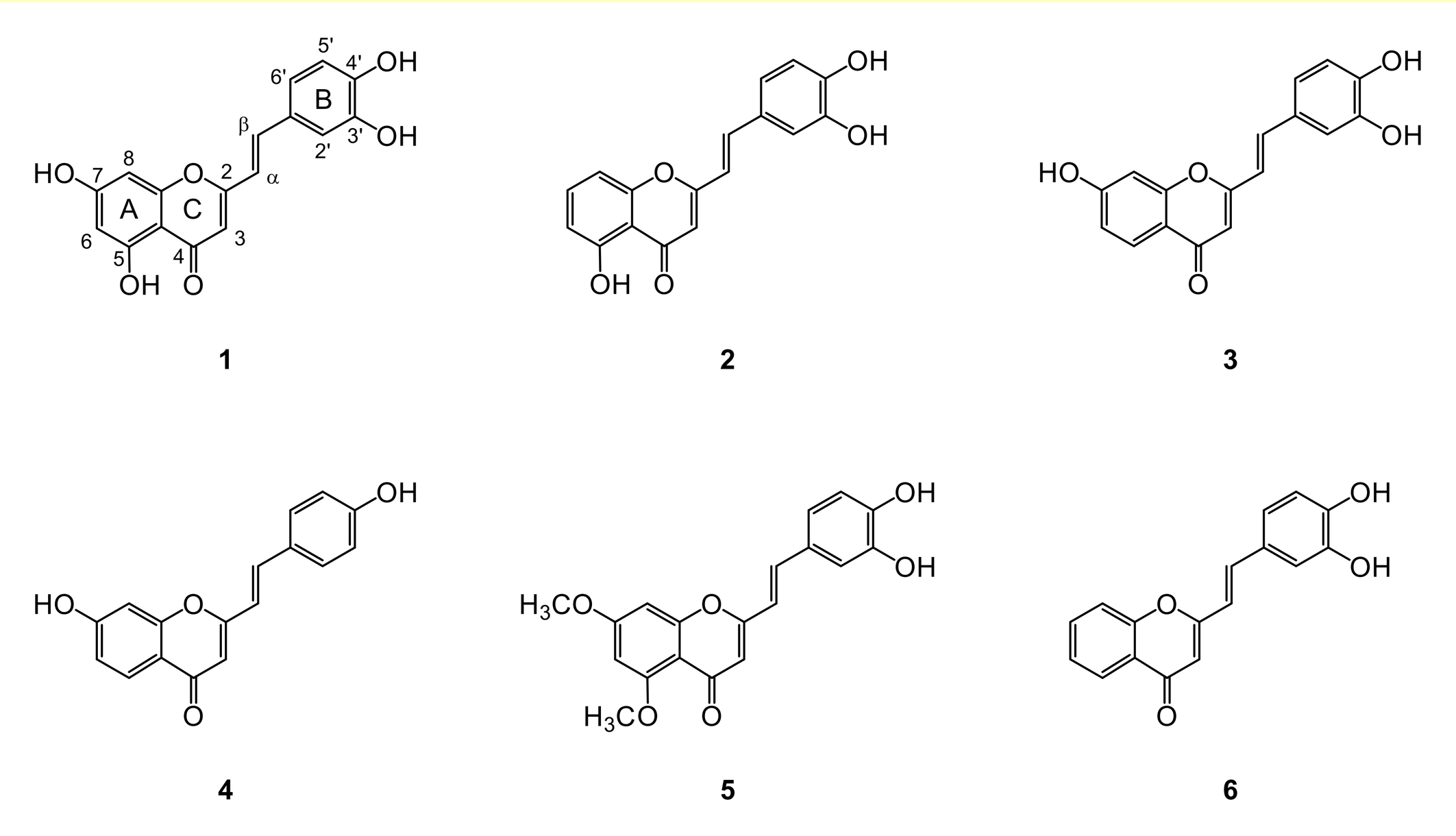


Figure 1*: Chemical structures of the tested 2-SC.*

Methods

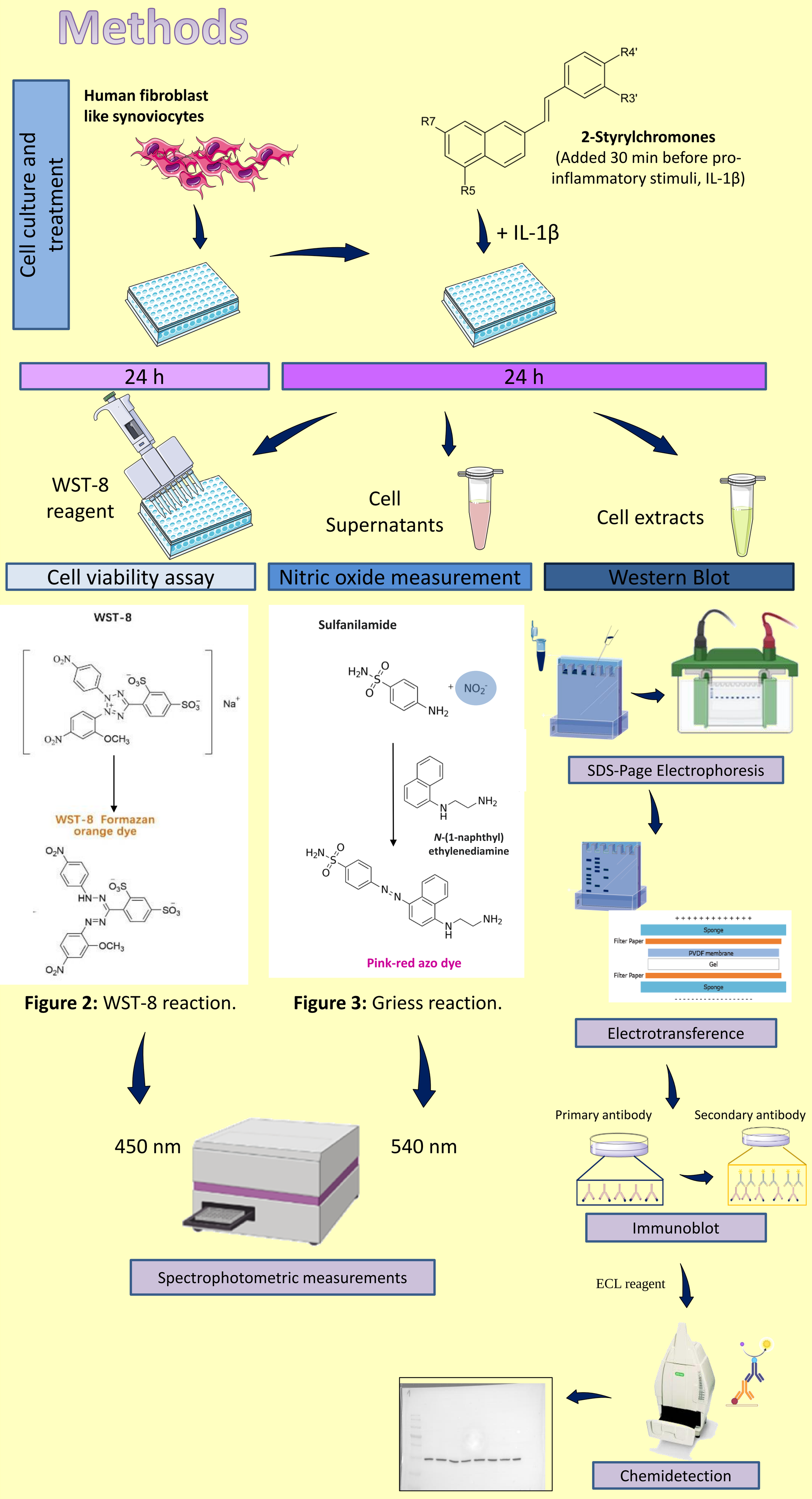


Figure 2: WST-8 reaction.

Figure 3: Griess reaction.

Results

Evaluation of cytotoxicity and selection of non-cytotoxic concentrations of 2-SC

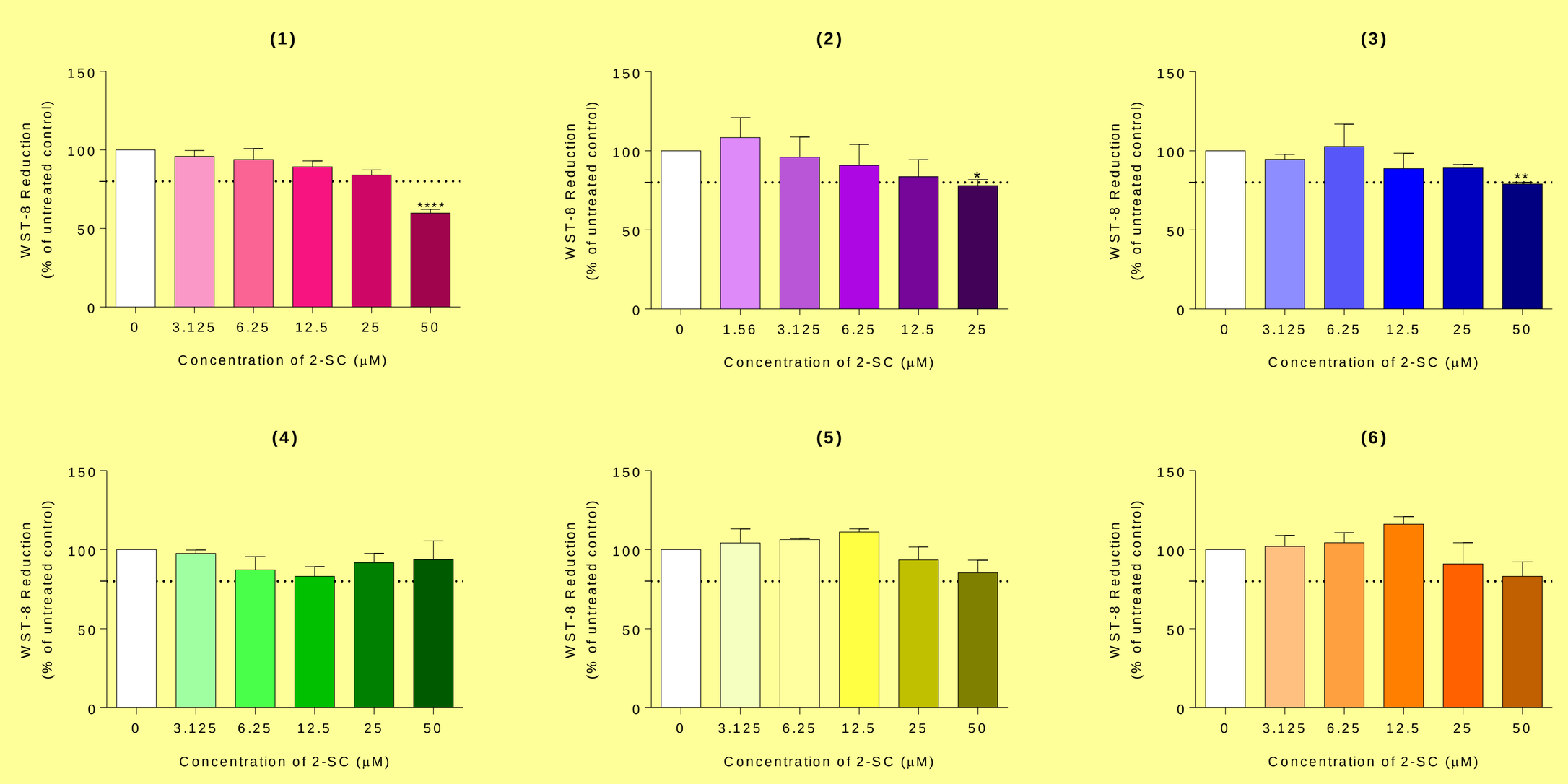


Figure 4*: Effect of 2-SC on the viability of HFLS. Cell viability was assessed in cell cultures treated with the indicated concentrations of the 2-SC for 24 h. Each column represents, at least, 4 independent experiments. The dotted line represents 80 % of the maximal absorbance below which cell viability is considered compromised. *p<0.05, **p<0.01, ****p< 0,0001 relative to the respective control (untreated) cells.*

Effects of 2-SC on IL-1β-induced nitric oxide production and iNOS protein expression

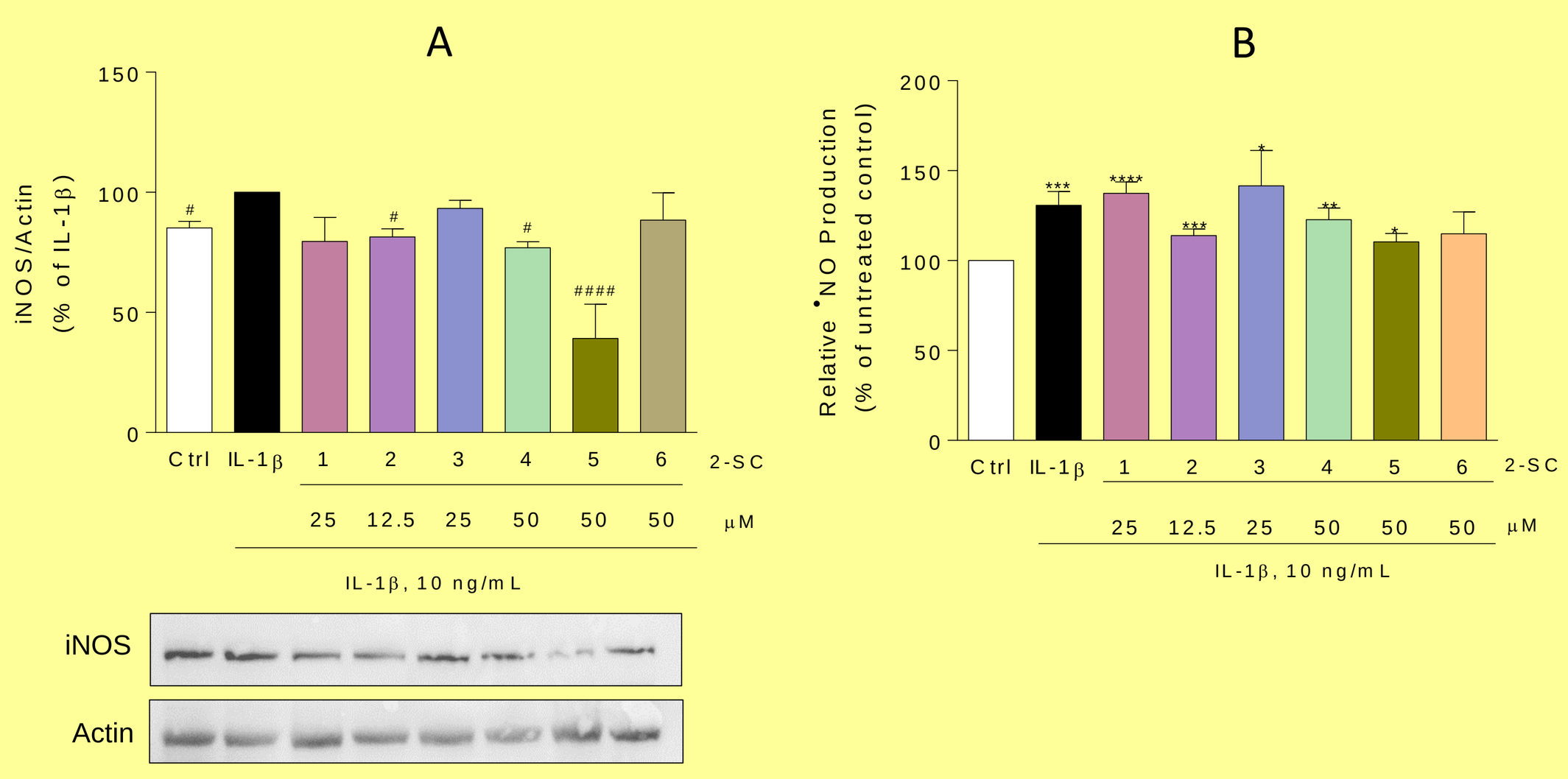


Figure 5*: Effect of 2-SC on IL-1β - induced iNOS protein expression (A) and •NO production (B) in HFLS. Cell cultures were left untreated (control, Ctrl) or treated with IL-1-β (10 ng/mL), for 24 h, following pre-treatment for 30 min with the indicated concentrations of the 2-SC. Each column represents the mean ± SEM of, at least, 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 relative to Ctrl. #p<0.05, ####p<0.0001 relative to IL-1β treated cells.*

Effect of 2-SC on IL-1β-induced NF-kB activation

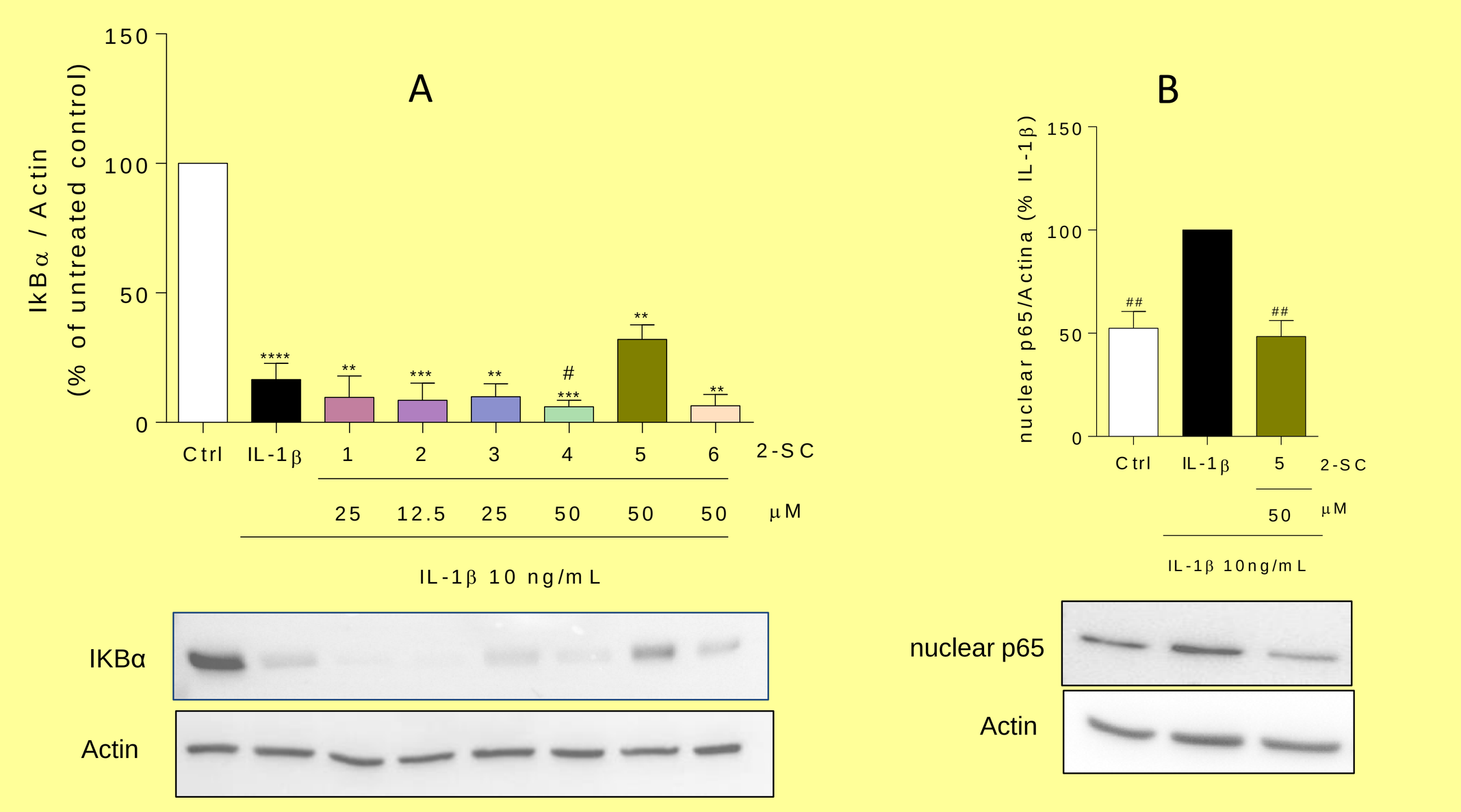
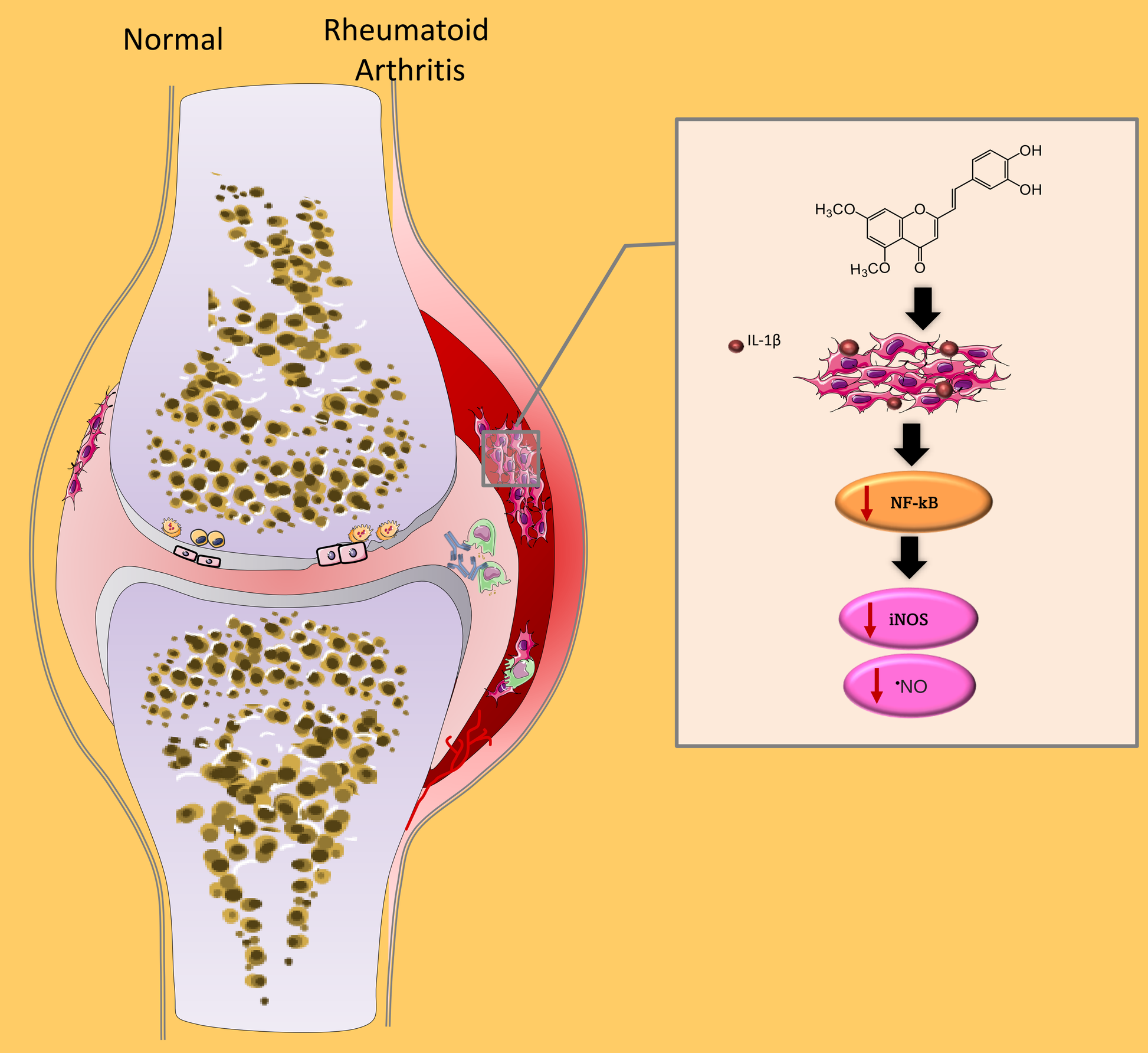


Figure 6*: Effect of 2-SC on IL-1β - induced NF-κB activation, evaluated as the levels of total IκB-α (A) and nuclear p65 (B) on HFLS that were left untreated (control, Ctrl) or treated with IL-1β (10 ng/mL), for 30 min, following pretreatment for 30 min with the indicated concentrations of the 2-SC. Each column represents the mean ± SEM of 3 independent experiments. **p<0.01, ***p<0.001, ****p<0.0001 relative to Ctrl. #p<0.05, ##p<0.01 relative to IL-1β treated cells.*

Discussion and conclusion

From the tested 2-SC, the one presenting two OCH_3 at C-5 and C-7 on A-ring and a catechol group on B-ring (**5**), significantly reduced iNOS expression and •NO production. These effects seemed to be partially mediated by the reversion of IL-1β - induced cytoplasmic IkBα disappearance and nuclear p65 increase, pointing the role of NF-kB in the process.

These findings may be of great value in the development of new 2-SC which should be further explored and carefully evaluated to reveal their full potential on the RA treatment.



Aknowledgments

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